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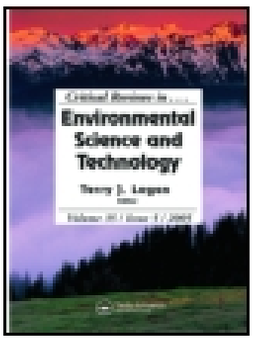

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Plastic ingestion by marine fish in the wild

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ABSTRACT

Marine plastic pollution has become a prominent environmental issue in the recent years. Plastic ingestion is of special concern, as its magnitude and consequences for marine organisms and potentially humans are still largely unknown. We reviewed 93 papers on plastic ingestion by wild marine fish published since 1972. Plastic ingestion was detected in 323 (65%) of 494 examined fish species, and in 262 (67%) of 391 examined commercial fish species. These proportions are likely greater, as a detailed analysis of the sampling effort and analytical methods used in the reviewed studies suggests an underestimation of plastic ingestion in some assessments. A significant positive relationship ($R = +0.845$, $p = 0.004$) was found between the sample size up to $N = 10$ and the detection of plastic ingestion. We also found significant differences in detection and frequency of occurrence (FO, %) of plastic ingestion among the three main types of analytical methods: naked-eye, microscopic analysis and chemical digestion. The chemical digestion method, which is also the most robust laboratory method, had the greatest detection (86%) and the highest FO (37.6 ± 0.6%). To avoid the underestimation of plastic ingestion in future work, we provided recommendations for sample sizes and laboratory analysis.

KEYWORDS

Contamination; gut content analysis; marine debris; marine plastic pollution; methodology; microplastics; sample size; seafood

1. Introduction

Although the mass production of plastics started only after WWII (Carpenter & Smith, 1972); these highly versatile synthetic materials soon found their way into countless applications and today, our everyday lives are unimaginable without plastics. The global production has increased from 1.5 million tonnes in 1950s to 348 million tonnes in 2017 (PlasticsEurope, 2010, 2018). Consequently, due to plastics overconsumption and waste mismanagement,

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the release and proliferation of plastics in natural environments was inevitable (Geyer, Jambeck, & Law, 2017; Law, 2017; Worm, Lotze, Jubinville, Wilcox, & Jambeck, 2017). As a severe environmental contaminant, plastics have been documented to cause numerous ecological problems, including the more frequently reported ingestion of plastics (Rochman, Browne, et al., 2016; Wilcox, Mallos, Leonard, Rodriguez, & Hardesty, 2016).

Based on the currently available reviews, the occurrence of plastic ingestion among marine organisms has been documented in over 200 species (Gall & Thompson, 2015; Kühn, Rebolledo, & van Franeker, 2015; Ryan, 2016) and is particularly common in sea turtles, marine mammals and seabirds (Kühn et al., 2015). With respect to invertebrates, microplastics have been found in beach worms (Gusmão et al., 2016), gooseneck barnacles (Goldstein & Goodwin, 2013), jellyfish (Macali et al., 2018), limpets, periwinkles, sponges, anemones, brittle stars, isopods (Karlsson et al., 2017), copepods (Desforges, Galbraith, & Ross, 2015), and even deep-sea invertebrates (Taylor, Gwinnett, Robinson, & Woodall, 2016), but also in species valuable to commercial and artisanal fisheries, such as mussels (Galimany, Ramon, & Delgado, 2009; De Witte et al., 2014), oysters (Van Cauwenberghe & Janssen, 2014), clams (Davidson & Dudas, 2016), shrimps (Devriese et al., 2015), lobsters (Welden & Cowie, 2016) and squids (Rosas-Luis, 2016). Apart from the commercial invertebrates, marine plastics and microplastics have been recovered from numerous commercial fish species as well, including sardines (Clupeidae) and anchovies (Engraulidae) (Compa, Ventero, Iglesias, & Deudero, 2018), sea bass (Moronidae), seabream (Sparidae) and flounder (Pleuronectidae) (Bessa et al., 2018), cod (Gadidae) (Foekema et al., 2013), mullet (Mugilidae) (Naidoo, Smit, & Glassom, 2016; Jabeen et al., 2017), mahi-mahi (Coryphaenidae) (Markic et al., 2018), swordfish (Xiphiidae) and tuna (Scombridae) (Romeo et al., 2015). Thus far, the maximum number of fish species reported to ingest plastic to date was 93, as reported by Ryan (2016). Uncovering plastic debris in seafood additionally raises great concern for human health (Santillo, Miller, & Johnston, 2017).

Ingestion of plastics can occur directly (primary ingestion) or indirectly (secondary ingestion), by ingesting prey which contain plastic. Secondary plastic ingestion is also referred to in the literature as the trophic transfer of microplastics (Au, Lee, Weinstein, van den Hurk, & Klaine, 2017). Some studies experimentally demonstrated the trophic transfer of microplastics and nanoplastics from lower to higher trophic levels in marine (Farrell & Nelson, 2013; Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014; Nelms, Galloway, Godley, Jarvis, & Lindeque, 2018) and freshwater organisms (Cedervall, Hansson, Lard, Frohm, & Linse, 2012). Evidence of trophic transfer has been confirmed in field subjects as well (Chagnon et al., 2018;

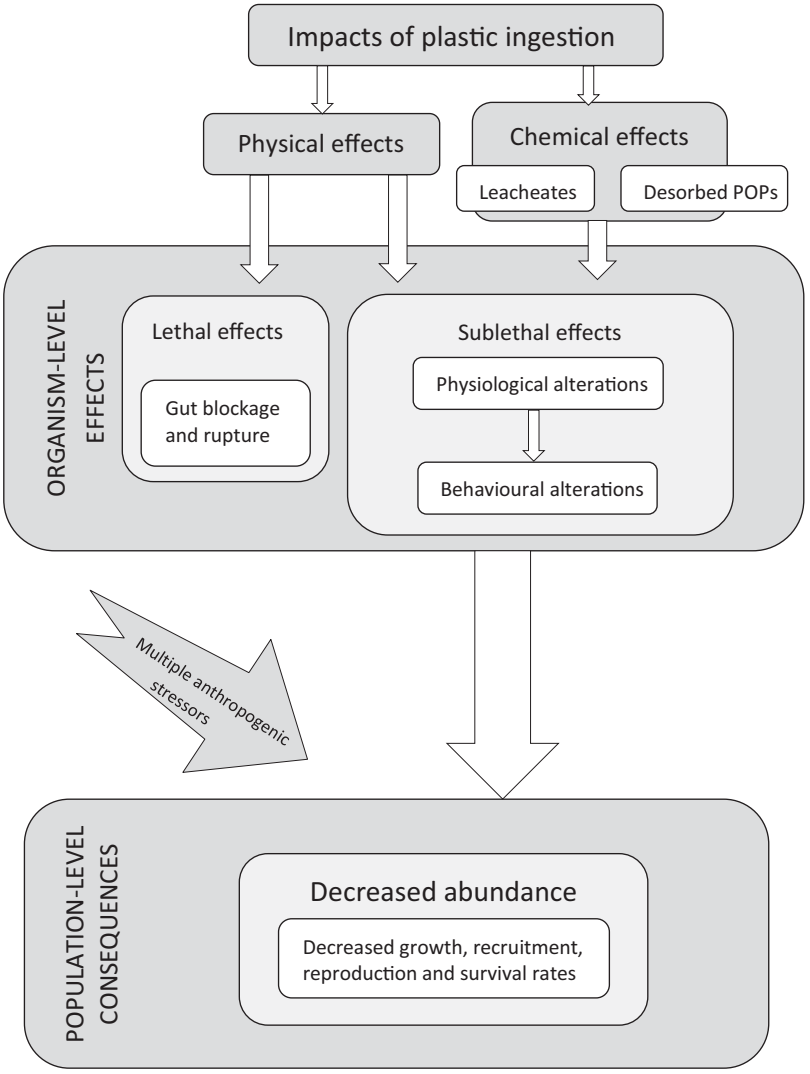

Figure 1. Conceptual diagram of potential impacts of plastic ingestion on marine animals, including the presence of other anthropogenic stressors (modified from Lavers, Bond, & Hutton, 2014).

Hipfner et al., 2018; Markic et al., 2018). Santillo et al. (2017) suggest that, even if organisms of lower trophic levels consume rather small amounts of microplastics, for the higher trophic levels predators, who predate on these organisms, it would mean much higher ingestion of microplastics over time.

1.1. Adverse effects of plastic ingestion on marine fish

Plastic ingestion has detrimental physical and chemicals effects on marine organisms (Figure 1). These include lethal and sub-lethal effects, of which

the latter is difficult to quantify, especially on a level higher than an individual organism (Kühn et al., 2015). Ingestion of plastic objects had been found to cause direct mortality by gut obstruction and perforation in numerous marine vertebrates, such as sea birds (e.g. Pierce, Harris, Larned, & Pokras, 2004), marine mammals (Puig-Lozano et al., 2018) and sea turtles (Wilcox, Puckridge, Schuyler, Townsend, & Hardesty, 2018). To our knowledge, direct mortality in wild fish caused by ingested plastic has not yet been described in published literature. However, several papers described ingestion of large pieces of plastic by fish, such as plastic cups in fish from English Channel (Anonymous, 1975), gumboots, paint roller and plastic bags in sharks from South Africa (Cliff, Dudley, Ryan, & Singleton, 2002), and various large fragments of hard and soft plastics (>1 cm) in fish from the North Pacific Ocean (Choy & Drazen, 2013; Jantz, Morishige, Bruland, & Lepczyk, 2013). These objects would most likely eventually cause death. Indirect physical impacts of micro- and nanoplastics on fish have been demonstrated experimentally, and include decreased mobility, feeding and growth, reduced body condition and overall performance (e.g. Critchell & Hoogenboom, 2018; de Sá, Luís, & Guilhermino, 2015). Except for plastics being found in the digestive tract of wild fish, several papers described accumulation of smaller size plastic particles in the gills (Collard, Gilbert, Eppe, et al., 2017; Karami, Golieskardi, Ho, Larat, & Salamatinia, 2017; Abbasi et al., 2018) and their translocation to liver and muscle tissue (Collard, Gilbert, Compère, et al., 2017; Abbasi et al., 2018; Akhbarizadeh, Moore, & Keshavarzi, 2018; Karami, Golieskardi, Ho, et al., 2017). In an experimental study, Mattsson et al. (2017) also demonstrated that plastic nanoparticles can pass through the blood-brain barrier and cause behavioral disorders in fish. Hence, physical effects of plastics depend on their size, and generally, plastics toxicity increases with dose and with the decrease of the particle size (Mattsson et al., 2017).

Other concerns relate to the introduction of various potentially toxic chemicals to fish via ingestion of marine plastics. Marine plastics, as well as other organic matter in the marine environment, adsorb anthropogenic compounds already present in the water, such as pesticides, fertilizers and industrial chemicals (e.g. PCBs, DDTs, PAHs, PBDEs), also known as persistent organic pollutants or POPs (Rochman, 2015; Anbumani & Kakkar, 2018). Furthermore, various chemicals are added to plastics during their production to change their properties (e.g. BPA, phthalates, PBDEs) and they can leach out of the material (Hermabessiere et al., 2017). Some organic compounds, such as styrene which is the building block of polystyrene, have been found to leach under certain conditions (Kwon et al., 2014). Although there is a growing consensus that marine plastics do not play a major role in the transfer of anthropogenic chemicals to marine

organisms and thus in potential harm (Koelmans, 2015; Koelmans, Bakir, Burton, & Janssen, 2016; Ziccardi, Edington, Hentz, Kulacki, & Driscoll, 2016; Burns & Boxall, 2018; Ogonowski, Gerdes, & Gorokhova, 2018; Paul-Pont et al., 2018), it has been demonstrated experimentally that the externally- and internally-bound chemicals, including their metabolites which are sometimes more detrimental than the parent compound (Geyer et al., 2000), can cause physiological alterations, including endocrine disruption (Rochman, Kurobe, Flores, & Teh, 2014), and histopathological alterations of liver (Rochman, Hoh, Kurobe, & Teh, 2013; Rainieri, Conlledo, Larsen, Granby, & Barranco, 2018) and intestines (Pedà et al., 2016). However, it is also likely that the exposure conditions in these experiments are not fully representative of those in the actual marine environment. Furthermore, it has been demonstrated recently that microplastics also can take up chemicals from gut fluids, thereby decreasing the chance of such adverse effects (Mohamed Nor & Koelmans, 2018). In conclusion, considering the exceptionally complex nature of marine plastics as an environmental contaminant, the evaluation of associated risks is a challenging task.

1.2. Aim of the study

As a response to the evident lack of synthesized literature on plastic ingestion by wild marine fish, and the burning issue of contamination of seafood by marine plastics and its potential consequences on human health, here we provide a global synthesis on the matter. The only literature review to date focused specifically on plastic ingestion by marine fish was published almost 30 years ago and it was based on eight published articles and one anecdotal evidence (Hoss & Settle, 1990). There are several reviews of a broader scope, covering the impacts of plastics on marine animals, which also included plastic ingestion by fish (Laist, 1997; Gall & Thompson, 2015; Kühn et al., 2015; Ryan, 2016). However, being a part of broad reviews, plastic ingestion specifically by wild fish was described succinctly, providing only more general information.

The aim of our study was to review and synthesize available information, summarize previous findings and update current knowledge on plastic ingestion by fish in the wild in more detail. More specifically, we intended to provide the updated number of wild marine fish species which were recorded to ingest plastic and to investigate why some species do and others do not ingest plastic, by examining the patterns in plastic ingestion with respect to habitat, feeding strategy and geographical distribution of examined species, as well as to critically review sampling and analytical methodology. In a recently published study, Hermsen, Mintenig, Besseling, & Koelmans (2018) reviewed 35 publications on plastic ingestion by marine

biota and evaluated their validity and reliability through a scoring system, highlighting, among others, the issue of small sample sizes, sampling, storing and laboratory analytical methods. A short review of the methods and results of 15 studies on plastic ingestion by wild fish was also provided by Cannon, Lavers, & Figueiredo (2016), as a part of their field study. Here, we looked more closely at whether sample sizes and analytical methodology affected the detection of plastic ingestion in fish. With detailed guidelines for future studies given by Hermsen et al. (2018), we also provided a list of recommendations, including the sample size calculations.

2. Methods

2.1. Literature review

We systematically reviewed 93 papers published between 1972 and 1 January 2019, which included 79 studies on plastic ingestion by wild fish, 9 fish diet studies and 5 plastic ingestion incidence reports. Databases and search engines used to search for papers on plastic ingestion by fish included: Google Scholar, Web of Science, Science Direct, BioOne and Wiley Online Library. Combinations of the following words were used for keyword search: marine fish, plastic ingestion, fish diet, microplastics, marine debris and anthropogenic debris.

In plastic ingestion studies, plastic is actively searched for by examining the gut content, while in the diet studies and incidence reports, the detection of plastics was incidental, which may lead to either underestimation or overestimation of plastic ingestion, respectively. For this reason, the diet studies and incidence reports were used only for extracting basic information (i.e. species names), while the plastic ingestion studies were used for more in-depth review, analysis of methodology and occurrence of plastic ingestion. From these 79 studies, we extracted the qualitative and quantitative data, descriptions and definitions of which are provided in Table 1. Additional information gathered from FishBase (Froese & Pauly, 2018), World Register of Marine Species (WoRMS Editorial Board, 2018), Marine Species Identification Portal (Marine Species Identification Portal, 2018), The IUCN Red List of Threatened Species (IUCN, 2018), and Food and Agriculture Organization of the United Nations (FAO, 2016, 2018) included: (i) fish taxonomy; (ii) habitat, (iii) trophic level; (iv) IUCN conservation status and (v) fisheries interest. This information is provided in Supplementary information.

For the species count, we included the species provided as *Genus* sp. only if they were the only species of that genus in the extracted dataset. Conversely, *Genus* sp. was excluded from the count if there were other examined species of the same genus. Pooled species (i.e. *Genus* spp.) were

Table 1. Information extracted from 79 plastic ingestion studies.

Type of data	Symbol (measurement unit)	Description
Species name		Species with and without record of plastic ingestion
Sample size	*N* (#)	Number of examined specimens per assessment of one species
Frequency of occurrence	FO (%)	Percentage of specimens in one assessment of one species which were found to contain plastic debris in their gastro-intestinal (GI) tract.
Plastic load	PL (piece per individual or pc ind^{-1}; mass per individual or g ind^{-1})	The quantity of plastic pieces per individual fish of one assessment, including only specimens which contained plastic
Methodology		Sampling and analytical procedure
Plastic size	(mm)	The size of recovered plastics in three size categories: <1 mm, 1–5 mm, >5 mm
Fish body length	(cm)	Fish body size (cm)
Geographic location		Study location/s

excluded as well. Since some species were examined multiple times in different studies, we use the term '*assessment*' to indicate one evaluation (i.e. measurement or observation) of plastic ingestion in one species. Assessment refers to examination of one species in one study. In studies where the same species was assessed in several distant locations (i.e. different regions) and the incidence of ingestion was given separately (e.g. Markic et al., 2018), these assessments were considered as separate assessments of the same species. Similarly, if one species was examined using different methodology in the same study, these were also considered as separate assessments of the same species (e.g. Anastasopoulou et al., 2018). It should also be noted that assessments have variable sample sizes. For example, Atlantic herring (*Clupea harengus*, Clupeidae) was assessed for plastic ingestion on three individuals ($N = 3$) in one study (Collard, Gilbert, Eppe, Parmentier, & Das, 2015), and on 566 individuals ($N = 566$) in another (Foekema et al., 2013).

2.2. Measurement units

The most commonly used measurement unit for plastic ingestion by fish is the frequency of occurrence of plastic ingestion (FO), also called ingestion rate in some studies. FO is expressed as the percentage (%) of individual fish which contained plastics in one assessment. Another measurement unit is plastic load (PL), the amount of plastic per fish, but it is less common and less definite than FO. PL is often expressed as the number of plastic bits per fish that ingested plastic (e.g. Boerger, Lattin, Moore, & Moore, 2010; Avio, Gorbi, & Regoli, 2015; Tanaka & Takada, 2016), where the specimens which did not contain plastic were excluded from the calculation. However, some authors expressed PL as the number of plastics per all specimens in the sample, with and without plastic (e.g. Vendel et al., 2017). In some studies, PL was also expressed as the

mass of recovered plastics per specimens with plastic, or per all specimens in the sample (e.g. Boerger et al., 2010; Jantz et al., 2013; Benjamin et al., 2014). Due to large inconsistency in reporting PL values, which were also often completely omitted, we used FO as our main measurement unit in table summaries, plots and statistical analyses, while PL data were sufficient only for demonstrating averages on a global level.

2.3. Data interpretation and statistical analyses

To express the measure of central tendency of FO across different groups of our interest (e.g. methodology) we used weighted averages based on the following formula:

$$X_w = \frac{\sum_{i=1}^{n} p_i}{\sum_{i=1}^{n} N_i} = \frac{p_1 + p_2 + \ldots + p_n}{N_1 + N_2 + \ldots + N_n}$$

where X_w is weighted mean, N is the sample size of an individual assessment (i.e. total number of examined specimens of one species in one assessment), p is the number of specimens per assessment which contained plastic. Furthermore, to obtain more representable weighted averages, they were calculated excluding the outliers (i.e. the outlying sample sizes for each group of interest). The reason for this is that weighted averages are susceptible to distortion by very large sample sizes (or the denominators in the above formula) (See Supplementary information for a more detailed explanation). Statistical analyses were performed in XLSTAT (v. Base 19.7). Spearman correlation test was used to examine the statistical dependence between two variables. Chi-square test with Monte Carlo method (5000 simulations) was used to test for significance in the differences between multiple proportions. Marascuilo procedure, which compares all pairs of proportions, was used to further identify which proportions were responsible for rejecting H_0. Chi-square and Exact Fisher's test were run on contingency tables to examine the association between two qualitative variables and a number of different categories within them. Furthermore, we also provided recommendations for minimal sample sizes required to obtain the estimated proportion of ingestion, using the following formula for sample size calculation for proportions (Milton, 1999):

$$N \geq (z/m)^2 * P(1-P)$$

where N is the sample size, z is the standard z-score extracted from the z-table for standard normal probabilities, m is the margin of error and P is the estimated population mean or the proportion of all examined fish

which contained plastic, based on the results of the review (i.e. P is the same value as the average global FO, except expressed as a proportion (0–1) instead of a percentage (0–100%). We provided minimal required sample sizes for 95% confidence level ($z = 1.96$), and we tentatively set up the margin of error to be 10% ($m = 0.1$) and 20% ($m = 0.2$), as two examples.

Furthermore, to assess research effort across different habitats, we broadly divided habitats into their horizontal (neritic, neritic-oceanic and oceanic) and vertical (benthic and demersal, benthopelagic, and pelagic) components. Neritic refers to coastal and oceanic to offshore waters. Benthic and demersal refers to habitats on or near the bottom, while pelagic habitats include surface waters. The main challenge was to assign the most appropriate habitat category to each species. To stay consistent, we used FishBase (Froese & Pauly, 2018). One horizontal and one vertical habitat category was assigned to each species. With respect to feeding, each species was assigned a trophic level and a trophic guild, also extracted from FishBase (Froese & Pauly, 2018). Trophic levels, which have values from 1 to 5, indicate common diet composition of a species and its position in the food web. The values increase proportionally with the position of the species in the food web, with herbivores occupying lower levels of the food web (lower trophic levels, 1–2), and top predators the highest (higher trophic levels, 4–5) (Froese & Pauly, 2018). Based on their common diet, each fish species was placed into one of the five trophic guild categories: pelagic predators (nektivores feeding on fish and squid), benthic predators (fish feeding on benthic vagile invertebrates and small fish), planktivores (fish eating zooplankton and phytoplankton, including large zooplankton such as jellyfish and salps), omnivores (fish feeding on plant and animal matter, including detritus), and grazers (benthic herbivores, corallivores, spongivores and feeders on other sessile organisms).

To display global research effort on plastic ingestion by wild marine fish, we created a bubble map in Geolytics, a free Google map tool (https://geo.sg/). The map was created using averaged FO as a single data point for each study, or multiple data points per study when the study included multiple distant locations (e.g. Bellas, Martínez-Armental, Martínez-Cámara, Besada, & Martínez-Gómez, 2016; Forrest & Hindell, 2018; Markic et al., 2018; Ory et al., 2018).

3. Results and discussion

3.1. Overview of studies considered

The first studies on plastic ingestion by fish were published in the early 1970s (Carpenter et al., 1972; Kartar, Abou-Seedo, & Sainsbury, 1976), but

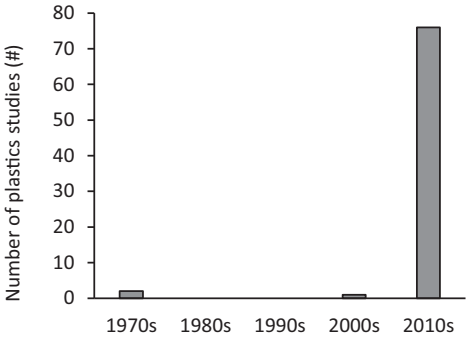

Figure 2. Research effort on plastic ingestion by fish over time. The graph includes 79 studies on plastic ingestion.

surprisingly research did not take off until the current decade (Figure 2). However, recently there has been a drastic increase in research effort as a response to the growing concerns about human health implications, with 29 studies on plastic ingestion by wild fish published in 2018 alone.

With respect to the temporal range of the studies, most studies were based on data collected in a brief period of time, on a single occasion (e.g. Tanaka & Takada, 2016; Bessa et al., 2018) or over several years (e.g. Chagnon et al., 2018; Hipfner et al., 2018; Kühn et al., 2018), while only four studies extended over a decade and longer (Cliff et al., 2002; Bernardini, Garibaldi, Canesi, Fossi & Baini, 2018; López-López et al., 2018; van der Hal, Yeruham, & Angel, 2018) (See Table S1). Regarding the spatial distribution of the studies, the pioneering studies and reports on this topic were limited to England (Anonymous, 1975; Kartar et al., 1976) and the east coast of the United States (Carpenter et al., 1972). At present, fish species from all main ocean regions have been examined for plastic ingestion.

In the first and only review specifically focused on plastic ingestion by marine fish, Hoss & Settle (1990) reported plastic ingestion in 22 wild marine species. More recently, plastic ingestion by fish has been reported in three reviews of a broader scope (i.e. impacts of plastics on marine biota, such as seabirds, sea turtles and marine mammals), in the following number of fish species: 50 (Gall & Thompson, 2015), 92 (Kühn et al., 2015) and 93 (Ryan, 2016).

In our updated review of 93 papers, plastic ingestion was recorded in 323 species (65.4%) out of a total of 494 examined marine fish species. All examined species belong to 33 orders and 137 families, with 451 bony (Osteichthyes) and 43 cartilaginous (Chondrichthyes) species. Of the 323 species with a record of plastic ingestion, 297 species were bony fish and 26 were cartilaginous (Table S2). As expected, the majority of assessed species belongs to Perciformes (Table 2), which is the most numerous fish order (Froese & Pauly, 2018).

Table 2. The most numerous fish orders assessed in 93 reviewed papers.

Order	No. of examined species	No. of species with plastic
Perciformes	228	157
Myctophiformes	34	21
Clupeiformes	28	21
Scorpaeniformes	26	13
Pleuronectiformes	23	16
Gadiformes	20	14
Carcharhiniformes	18	12

3.2. Sampling methods and sample sizes

3.2.1. Sampling methods

In accordance with a wide range of fish habitats and behavior, collection methods varied considerably from one study to another (Table S1). The most common method of gathering samples was collection from oceans and seas by various nets (Lusher, McHugh, & Thompson, 2013; Collard et al., 2015; Alomar & Deudero, 2017), longlines (Anastasopoulou, Mytilineou, Smith, & Papadopoulou, 2013; Jantz et al., 2013) and hook-and-line methods (Gassel, Harwani, Park, & Jahn, 2013; Phillips & Bonner, 2015). In more recent studies, the samples were often collected from fish markets and wharves, especially when the study focused on plastic ingestion by commercial fish and its potential impacts on humans (e.g. Neves, Sobral, Ferreira, & Pereira, 2015; Rochman et al., 2015; Jabeen et al., 2017; Markic et al., 2018).

3.2.2. Sampling effort

The number of examined species per plastic ingestion study showed a strong variability, from just one species (e.g. Battaglia et al., 2016) to 69 species (Vendel et al., 2017), with an average of 8.7 ± 1.4 species per study. Over 100 species were assessed multiple times in different studies from various locations. Thus, the overall number of assessments in the reviewed studies differs from the total number of examined species. In plastic ingestion studies, there were altogether 650 assessments of 475 species (Table S2) (note that the diet studies and incidence reports were excluded here).

Sample sizes (N) across plastic ingestion studies ranged from one to 25,914 individual fish per one assessment of one species. Species were usually not pooled and one assessment included only one species. In long-term monitoring studies (e.g. Cliff et al., 2002; López-López et al., 2018), sample sizes often exceeded 1,000 specimens per species. Conversely, in a number of studies where fish guts were obtained opportunistically, sample sizes of some assessments were very small ($N < 10$) (e.g. Anastasopoulou et al., 2013; Neves et al., 2015) (Table S2). Additionally, sample sizes were not provided in some papers (e.g. Steer, Cole, Thompson, & Lindeque, 2017).

3.3. Laboratory analytical methods

3.3.1. Detection and isolation of plastics

Analytical methods used to detect and isolate plastics from fish guts are quite diverse as well (Table S1). However, they could be grouped into three categories: (i) visual examination of the gut content by naked eye (hereafter called Method 1) (e.g. Cliff et al., 2002; Cartes, Soler-Membrives, Stefanescu, Lombarte, & Carrasón, 2016); (ii) visual examination of the gut content by an optical microscope (Method 2) (e.g. Anastasopoulou et al., 2013; Lusher et al., 2013; Bråte, Eidsvoll, Steindal, & Thomas, 2016) and (iii) digestion of the gut content with subsequent filtration and microscopic analysis (Method 3) (e.g., Foekema et al., 2013; Avio et al., 2015; Rochman et al., 2015; Mizraji et al., 2017). Among the studies that provided information on methodology (74), the most common method was Method 2, applied in 48% of the studies, followed by Method 3 and Method 1 used in 40% and 12% of the studies, respectively (Figure 3). In one study, both Method 2 and Method 3 were used for sample processing (Anastasopoulou et al., 2018).

In the recent studies, Method 3, involving physical and chemical isolation of plastic debris from the rest of the gut content, has generally become more common. Procedures under Method 3 include: (i) chemical digestion of the gut content using bases such as KOH (Foekema et al., 2013; Rochman et al., 2015) and NaOH (Bellas et al., 2016), acids such as HNO_3 (Collard et al., 2015), or oxydising agents such as H_2O_2 (Avio et al., 2015) and NaClO (Collard et al., 2015), (ii) enzymatic digestion (Karlsson et al., 2017), and (iii) filtration (Foekema et al., 2013; Avio et al., 2015). The gut content is digested to facilitate the isolation of plastics, after which the remaining liquid is filtered and the filters are examined under a microscope. A more detailed description of various digestion methods can be found in Lusher, Welden, Sobral, & Cole (2017).

In several studies, analytical methods included rinsing of the gut content out of the digestive tract and its filtration, but without chemical digestion (e.g. Cannon et al., 2016; Peters, Thomas, Rieper, & Bratton, 2017; Kühn et al., 2018; Nelms et al., 2018). The efficiency of plastics recovery by filtration, without prior digestion, greatly depends on the amount of the gut content (pers. obs.). A set of filters with different mesh sizes (e.g. Cannon et al., 2016; Peters et al., 2017) helps sort the gut content by size, which facilitates the subsequent microscopic analysis of the filters; however, the efficiency still depends on the amount of the gut content left on the filters. Similarly, staining of the gut content (e.g. with rose bengal, Davison & Asch, 2011), for visual separation of plastics from the rest of the content, is successful only if ingested plastics were not biofouled (pers. obs.). Otherwise, the stain dyes the biological layer around the plastic together with other biological material in the gut content. Even though the

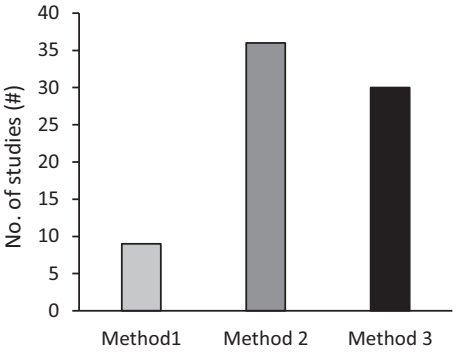

Figure 3. Number of plastic ingestion studies per method (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis).

efficiency of these methods might be greater than microscopic analysis alone, we believe they are still less efficient than methods which include chemical or enzymatic digestion and are specifically designed to isolate plastic from the gut content. Thus, to stay consistent, we included them in the Method 2 group.

Most studies included in the Method 3 group provided the mesh size of the filters used to filter the liquid remaining after the digestion of the gut content. The maximum reported mesh size was 250 µm (Lusher, O'Donnell, Officer, & O'Connor, 2016). Rochman et al. (2015) did not specify how the remaining liquid was analyzed; however, they noted that plastics over 500 µm were included in the report. In two other studies, filtering was not performed, but the digested content was poured into a Petri dish and examined under a microscope (i.e. Tahir & Rochman, 2014; Tanaka & Takada, 2016). Regardless of these studies missing the final filtration step, they were included in the Method 3 group because of the gut content digestion step.

3.3.2. Polymer characterization

Polymer characterization methods, such as Fourier transform infrared spectroscopy or FTIR (e.g. Foekema et al., 2013; Tanaka & Takada, 2016; Güven, Gökdağ, Jovanović, & Kideyş, 2017), and Raman spectroscopy (e.g. Collard et al., 2015), have become a common tool for the determination of the chemical composition of plastic debris found in biotic and abiotic samples. Polymer characterization is applied to either verify whether suspected particles were actually synthetic polymers (e.g. Wesch, Barthel, Braun, Klein, & Paulus, 2016; Güven et al., 2017), or to determine the overall composition of the extracted particles (Avio et al., 2015; Jabeen et al., 2017; Ory, Sobral, Ferreira, & Thiel, 2017), or both. Since polymer characterization can be a time-consuming and cost-prohibitive analysis, most authors

used only a subsample of recovered plastics (e.g. Güven et al., 2017; Jabeen et al., 2017). More seldom, all extracted particles were analyzed (Tanaka & Takada, 2016). Polymer characterization was applied in 42 studies, of which 40 studies used FTIR and two Raman spectroscopy. All but one (Carpenter et al., 1972) of these studies were published in the 2010s.

3.4. Methodological bias

Of the 475 fish species examined in plastic ingestion studies, plastic ingestion was not recorded in 171 species. To investigate the possibility of non-occurrence of ingestion in these species as a result of ecological differences, we compared the two groups (i.e. the species which ingested plastic and the species which did not ingest plastic) with respect to the feeding strategies of examined species. Both groups consist of very similar proportions of five categories of trophic guilds (Figure 4). We performed Chi-square and Exact Fisher's test on contingency tables to examine the association between the two groups (Without plastic and With plastic) and the proportions of fish species in each feeding category that the two groups consist of. The test showed that the groups are independent (Chi-square $p = 0.508$; Fisher's test $p = 0.546$), which indicates that the occurrence and nonoccurrence of plastic ingestion in these species was not associated with the feeding strategy.

We took a further look at the sampling effort and analytical methodology. We found that numerous assessments which did not detect plastic ingestion were done on small sample sizes ($N < 10$). There were 650 assessments of plastic ingestion in total, out of which the sample size was not given for seven assessments. Of the remaining 643 assessments, 258

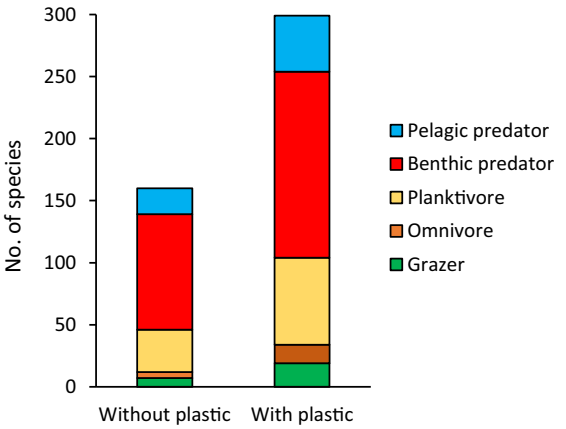

Figure 4. The number of species across different categories of trophic guilds with respect to plastic ingestion. 'Without plastic' refers to the group of species in which plastic ingestion was not detected, and 'With plastic' refers to species with detection of plastic ingestion.

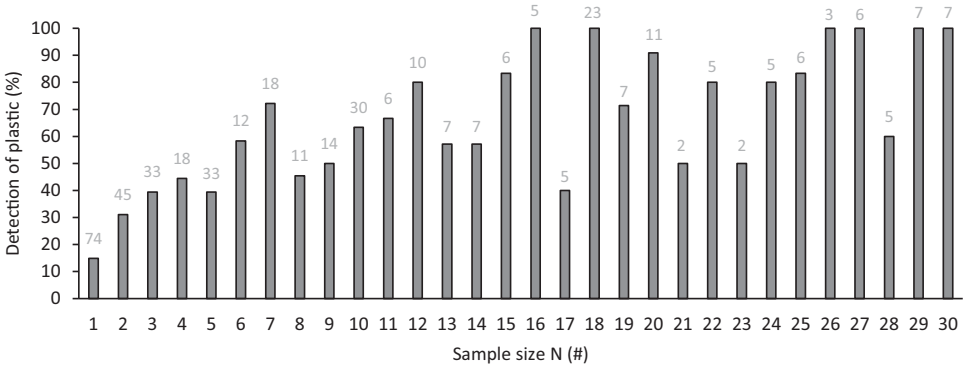

Figure 5. Frequency distribution of plastic detection across increasing sample sizes. Detection of plastic is expressed as a percentage of assessments which detected plastic in at least one examined specimen. Since data for some sample sizes $N > 30$ are lacking, we limited our display to only $N = 30$. Gray numbers above the bars present the number of assessments completed with the same sample size.

assessments (40.1%) were completed on less than 10 specimens per assessment ($N < 10$), and just over a third of these 258 assessments (35.3%) detected plastic ingestion. However, we found that as the sample sizes of the assessments increase, the detection of plastic ingestion increases as well (Figure 5). Note that the detection here refers to the percentage of assessments which detected plastic ingestion and were completed with the same sample size. For example, there were 74 assessments of plastic ingestion (the gray number above the first bar on the graph in Figure 5) which were performed on a single fish ($N = 1$), and of these 74 assessments, only 11 assessments detected plastic ingestion (i.e. detection of 15%). When the sample size increased to five individual fish per assessment ($N = 5$), 13 out of 33 assessments detected plastic ingestion (i.e. detection of 39%). The detection should not be confused with frequency of occurrence, as the detection is used here only to demonstrate the percentage of assessments in which plastic ingestion was detected, while FO is the percentage of individuals in one assessment which ingested plastic. We found a significant positive relationship between the detection of plastic ingestion and sample sizes up to $N = 10$ (Spearman's rank correlation, $R = + 0.845$, $p = 0.004$). Extending the correlation analysis on assessments with $N > 10$ would not have been robust, due to the low number of assessments in each N group (Figure 5, gray numbers). Thus, the absence of evidence of plastic ingestion in the assessments with $N < 10$ is likely, at least partly, to be an artifact of too small sample sizes.

In addition to sampling effort, the detection of plastics also seems to be dependent on analytical methods used for processing samples. We compared the detection and non-detection of plastic ingestion across the three different methods (Method 1, 2 and 3) and sample size groups, and it is

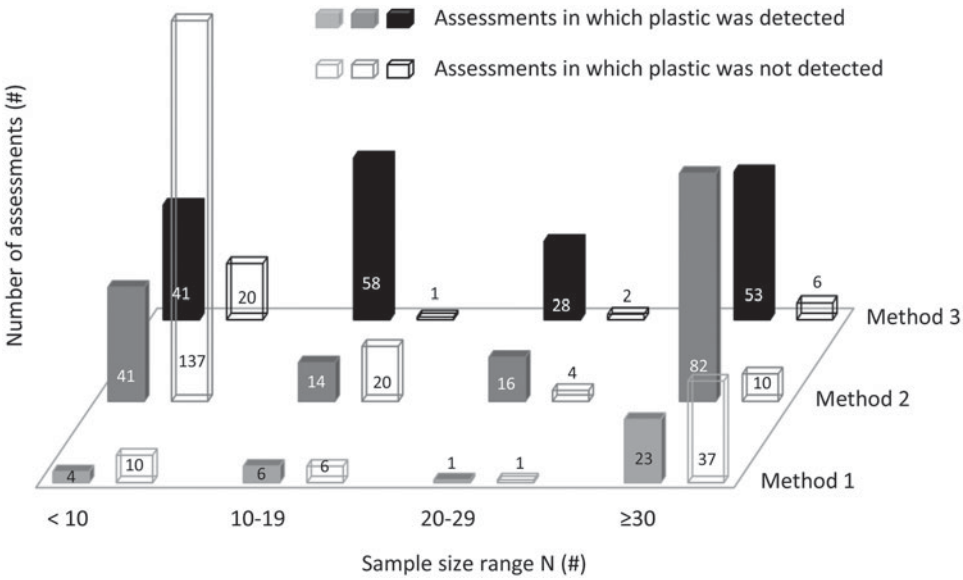

Figure 6. Detection of plastic with respect to different sample sizes and methods used (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis). Full bars present assessments in which plastic ingestion was detected, and the empty ones present assessments in which plastic ingestion was not detected.

evident that Method 3 has generally much higher detection than Method 1 and 2, even with lower sample sizes ($N < 10$ and $N = 10$–19) (Figure 6). The detection of Method 2 is particularly low in assessments with $N < 10$, but it increases in assessments with sample size $N > 20$, while the detection of Method 1 stays low even with increased sample sizes. Overall, the lowest detection (23%) occurred in 178 assessments done with Method 2 and $N < 10$, completed in 12 different studies (Figure 6, first two bars of Method 2).

With respect to differences in methodology, we tested the following two hypotheses: H_0: there is no significant difference in detection among methods, and H_0: there is no significant difference in FO among methods; using chi-square test for multiple proportions with Monte Carlo method (5000 simulations) and Marascuilo procedure. In both cases, we found significant difference ($p < 0.001$) and rejected the null hypotheses. Marascuilo procedure indicated that the detection was significantly different between Methods 1 and 3, and Methods 2 and 3, but there was no difference between Methods 1 and 2 (Figure 7a). In contrast, the differences in FO were statistically significant among all three methods (Figure 7b). FO obtained by Method 1 ($0.2 \pm 0.01\%$) were much lower than FO in Method 2 ($18.6 \pm 0.5\%$) and Method 3 ($37.6 \pm 0.6\%$), which were also significantly different from each other.

In several studies, where a large number of fish species was examined, with generally small sample sizes ($N < 10$) and using Method 2, the

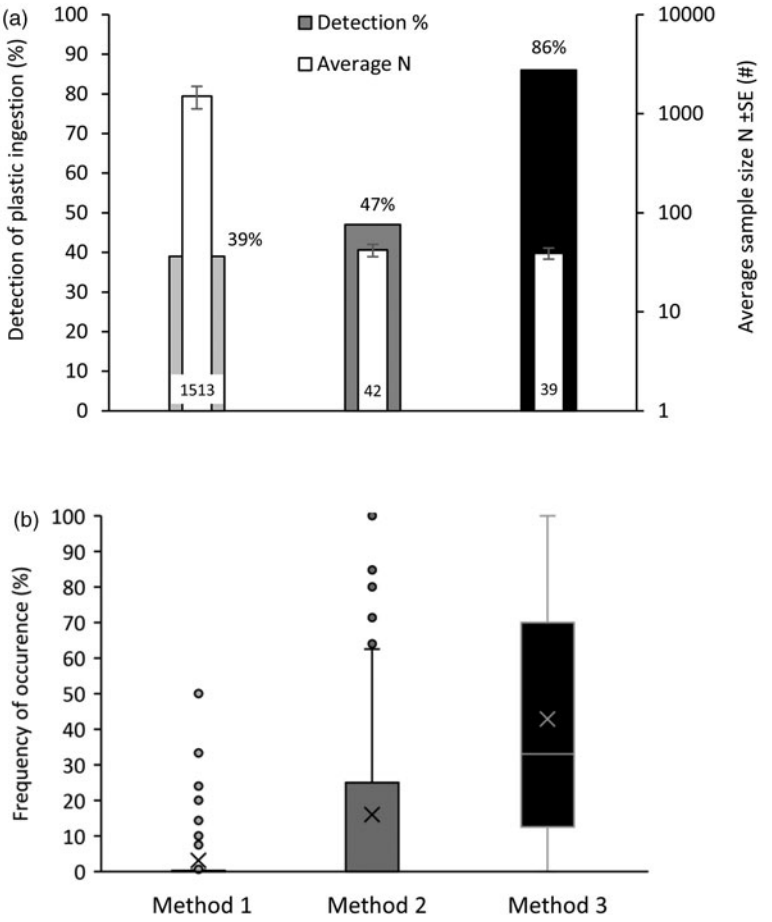

Figure 7. Difference in a) detection of plastic ingestion (% of assessments which detected plastic ingestion) and average samples sizes; and b) frequency of occurrence of plastic ingestion (FO) with respect to analytical methods (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis).

detection of plastic ingestion was particularly low (i.e plastic was found in less than a third of assessments) (Table 3). Considering that the studies covered a wide range of species collected from different regions, assuming there is a minimal chance of nonoccurrence of ingestion due to species-specific and location-specific factors, we suggest that the detection of plastic ingestion in these studies was low due to small sample sizes. For example, Vendel et al. (2017) examined 69 species of fish, collected in two tropical Brazilian estuaries, and 42 species were assessed with $N < 10$, out of which plastic ingestion was detected in only 3 species. The remaining 27 species were assessed with sample sizes ranging from 10 to 405 specimens and plastic ingestion was found in 21 species (Figure 8). In order to increase the power of their statistical analyses, the authors used only the results

Table 3. Studies with a large number of assessments (i.e. ≥20 assessments) using Method 2 and generally small sample sizes, resulting in low detection of plastic ingestion (less than 1/3 of assessments).

Study	No. of assessments	No. of assessments with non-detection	No. of assessments with non-detection and $N < 10$
Anastasopoulou et al., 2013	26	21	12
Davison and Asch 2011	27	19	18
Pegado et al., 2018	46	32	29
Vendel et al., 2017	69	45	39
Total	168	117	98

Note: Underestimation of plastic ingestion is suspected.

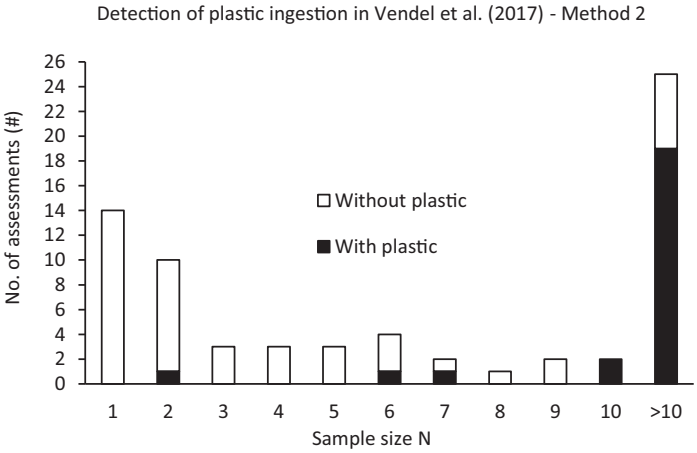

Figure 8. Detection of plastic ingestion in 69 species of fish which were examined with variable sample sizes in one study where Method 2 was applied (microscopic analysis) (Vendel et al., 2017).

obtained on the 27 assessments with $N \geq 10$, recognizing that the lower sample sizes are not robust enough to reliably detect plastic ingestion. Additionally, the results of this study only further confirm our previous finding that the detection increases with sample sizes (Figure 5).

Regarding the size of ingested plastic, ICES (2015) recommends visual examination only for meso- (5–25 mm) and macroplastic (>25 mm), while analysis on microplastics should include tissue digestion. With some difference in size categorization, Anastasopoulou et al. (2018) performed an analysis of macro- and micro-plastics, using Method 2 to examine the fish for macroplastics (>1 mm) and Method 3 for microplastics (<1 mm). They found much higher FO assessing microplastics (57.4%) than macroplastics (11.1%), corroborating that the Method 2 is poorly tailored for detecting microplastics. In the studies reviewed here, the sizes of extracted plastics, provided in 44 studies, also varied with respect to methodology, with smaller microplastics (<1 mm) being mainly isolated with Method 3, plastics from 1–5 mm were mainly found

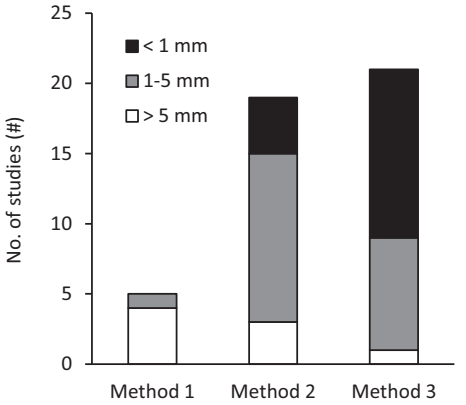

Figure 9. Sizes of plastic debris recovered from examined fish with respect to different analytical methods (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis), provided in 44 studies on plastic ingestion by fish.

in studies using Method 2, while larger plastics (>5 mm) were more common in studies using Method 1 (Figure 9). Size proportions of recovered plastic debris suggests that Method 1 readily detects large plastics while underestimating microplastics, but not that fish examined with Method 1 mainly contain large plastics.

Furthermore, it also seems that the choice of analytical method depends on the body length of examined fish, and presumably the amount of the gut content, and it is most likely a matter of practicality. Since the sizes of fish body length were provided in various units, such as standard length, fork length, precaudal length, total length, or it was not specified, direct comparison of these data would not be valid. However, we found that naked-eye visual examination (Method 1) was generally applied for large species, such as sharks (Cliff et al., 2002) and lancetfish (*Alepisaurus ferox*, Alepisauridae) (Jantz et al., 2013), while Method 2 and 3 were often used for smaller species, such as Myctophidae (e.g. Davison & Asch, 2011; Wieczorek et al., 2018), Clupeidae (Ory et al., 2018; Anastasopoulou et al., 2018), or juveniles and larvae (Steer et al., 2017; Vendel et al., 2017). Method 3 had not been commonly used for larger specimens, probably because it is impractical, expensive and time-consuming to chemically digest large quantities of the gut content of sharks and other large species. Conversely, Method 1 is not commonly used for examining a digestive system of minute specimens because it would leave out microplastics undetectable by naked-eye.

In addition, we looked at whether examining the entire digestive system was more common than examining the stomach content alone, and whether it yielded greater FO. In 51 studies, entire gastrointestinal tracts were analyzed, in 24 only the stomachs, and in four studies stomachs and

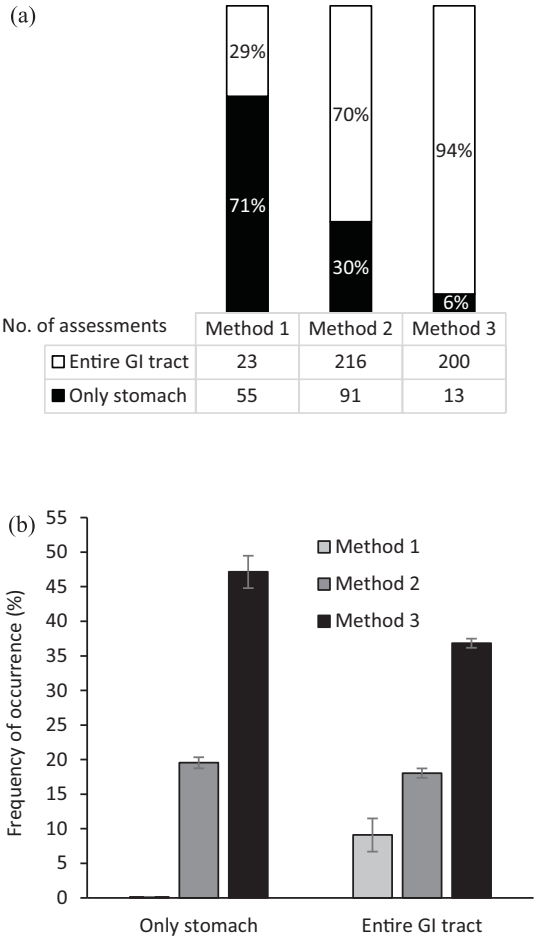

Figure 10. With respect to the part of the gastrointestinal (GI) tract examined, a) proportions (presented on the graph) and numbers (provided in the table below) of assessments, and b) frequency of occurrence, are graphically displayed for each of the three analytical methods (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis).

intestines variably, depending on the sample availability. When excluding the latter category, for a simpler demonstration, most assessments done with Method 1 examined only the stomachs, while the great majority of assessments using Method 2 and 3 included the stomach and intestines in the analysis (Figure 10a). With respect to the frequency of occurrence of plastic ingestion, greater FO was obtained for Method 1 when examining the entire GI tract (9.1 ± 2.4%) than examining only the stomach (0.1 ± 0.01%). Conversely, using Method 3, the FO is lower in the assessments of the entire GI tract (36.8 ± 0.7%) than in the assessments of the stomach alone (47.1 ± 2.3%). With respect to Method 2, the FO of assessments of the stomach alone (19.5 ± 0.8%) and the assessments of the entire GI tract (18 ± 0.7%) are similar. Due to insufficient data for some groups,

we did not perform statistical analysis. However, although our data suggest that examining the entire GI tract does not necessarily result in greater FO than when examining the stomach alone, Jabeen et al. (2017) highlighted the importance of examining the entire GI tract, and not only the stomach, as they found more plastic in the intestines than stomach of five out of 11 examined species. They also demonstrated that fish with more complex digestive tracts retain more plastics.

With respect to the type of plastic debris recovered from the examined specimens, there is also a potential bias, as some studies excluded fibers due to the possibility of airborne contamination. Davison and Asch (2011) excluded only small fibers, while several other studies excluded fibers entirely (e.g. Foekema et al., 2013; Avio et al., 2015) (Table S1). In other studies, where the fibers were included, the authors often used laboratory blanks as contamination controls (e.g. Rochman et al., 2015). Since the information provided on plastic type is quite inconsistent throughout the studies, there is no way of knowing in which studies the contamination potentially occurred, and a more in-depth analysis would not be reliable. This topic is also covered in Hermsen et al. (2018) in more detail. As a general overview, the type of recovered plastic objects and fragments varied from whole objects (Kartar et al., 1976; Cliff et al., 2002), filaments or fibers (Dantas, Barletta, & da Costa, 2012; Lusher et al., 2013; Neves et al., 2015; Rochman et al., 2015), plastic spherules (Carpenter et al., 1972; Kartar et al., 1976; Miranda and de Carvalho-Souza, 2016) and fragments (i.e. broken-down plastic particles of unknown origin; Boerger et al., 2010; Avio et al., 2015). The most commonly found were fibers, followed by fragments.

Finally, there is most likely a certain bias associated with the species-specific and location-specific occurrence of ingestion. For example, Atlantic herring was found to have low frequency of ingestion (FO $<$ 2%) in four studies conducted in the North Atlantic, while European pilchard (*Sardina pilchardus*, Clupeidae) examined in five studies in the Mediterranean exhibited FO from 15% to 57% (Table S4). Potentially Atlantic herring is less prone to plastic ingestion, or plastic debris was less available in the habitat of these individuals. However, the frequency of occurrence of plastic ingestion in European pilchard was generally much lower in studies completed with Method 2 (FO = 15–19%) than Method 3 (FO = 19–57%). We examined the occurrence of plastic ingestion in 17 species which were assessed multiple times (four times and over) in 35 studies (Table S4), and we found that the patterns in detection were very similar to the patterns in Figure 6 and that the majority of the assessments which did not detect plastic were completed with Method 1 and 2, particularly with sample sizes of $N < 10$ and Method 2. Furthermore, the frequency of occurrence in these 17 species obtained with the three methods also closely follows the FO in the

graph in Figure 7b (Table S4). Therefore, alongside the difference in environmental concentrations of marine plastics at the time of sample collection, which we do not have information for, and fish biology and ecology, we finally conclude that, due to methodological deficiency, plastic ingestion was underestimated in some species.

3.5. Plastic ingestion in spatial and temporal context

3.5.1. Global and regional occurrence of plastic ingestion in fish

Based on data obtained in 199 assessments of 169 species of fish using Method 3, on a global level, plastic ingestion occurs in about a third of individual fish (FO = 37.6 ± 0.6%) (i.e. the same value provided earlier as the FO obtained by Method 3), which on average ingest over two pieces of plastic per fish (PL = 2.6 ± 0.2 pc ind^{-1}). Due to the likely strong impact of methodological variability, the data obtained with Method 1 and Method 2 were excluded from these calculations. However, although not suitable for averaging FO and PL, the data from all three method categories may be used to demonstrate regional differences in terms of research effort. Most research is focused in the North European and Mediterranean marine waters (Figure 11a, Table 4), dominantly applying Method 3 (Figure 11b). Furthermore, it is also evident that research is greatly lacking in the North American, African, Asian and Australian waters, as well open ocean waters in general.

3.5.2. Temporal trends in plastic ingestion by fish

Of the four long-term studies on plastic ingestion by fish (Table S1), only two investigated temporal changes in the occurrence of plastic ingestion. van der Hal et al. (2018) reported an increase of plastic ingestion in siganids from Israel waters from 10% in the 1960s to about 80% from the late 1990s to the present. Conversely, Cliff et al. (2002) found no increase in plastic ingestion in tiger sharks (7.5%) (*Galeocerdo cuvier*, Carcharhinidae) from 1978 to 2000. These studies do not provide sufficient information to deduce on temporal trends of plastic ingestion by marine fish. Other studies on plastic pollution and plastic ingestion also provide inconclusive results, despite the constant increase of plastic production (PlasticsEurope, 2018), which presumably results in increasing trends in plastic pollution. Plastic ingestion by sea turtles (Schuyler, Hardesty, Wilcox, & Townsend, 2014) and sea birds (Avery-Gomm et al., 2012; van Franeker & Law, 2015) in time does not demonstrate any specific temporal trends. Similarly, the trends of plastics concentrations in the marine environment are also ambiguous (Law et al., 2010, 2014; Ribic, Sheavly, Rugg, & Erdmann, 2012; Nelms et al., 2017). Cózar et al. 2014 suggested that plastics concentrations

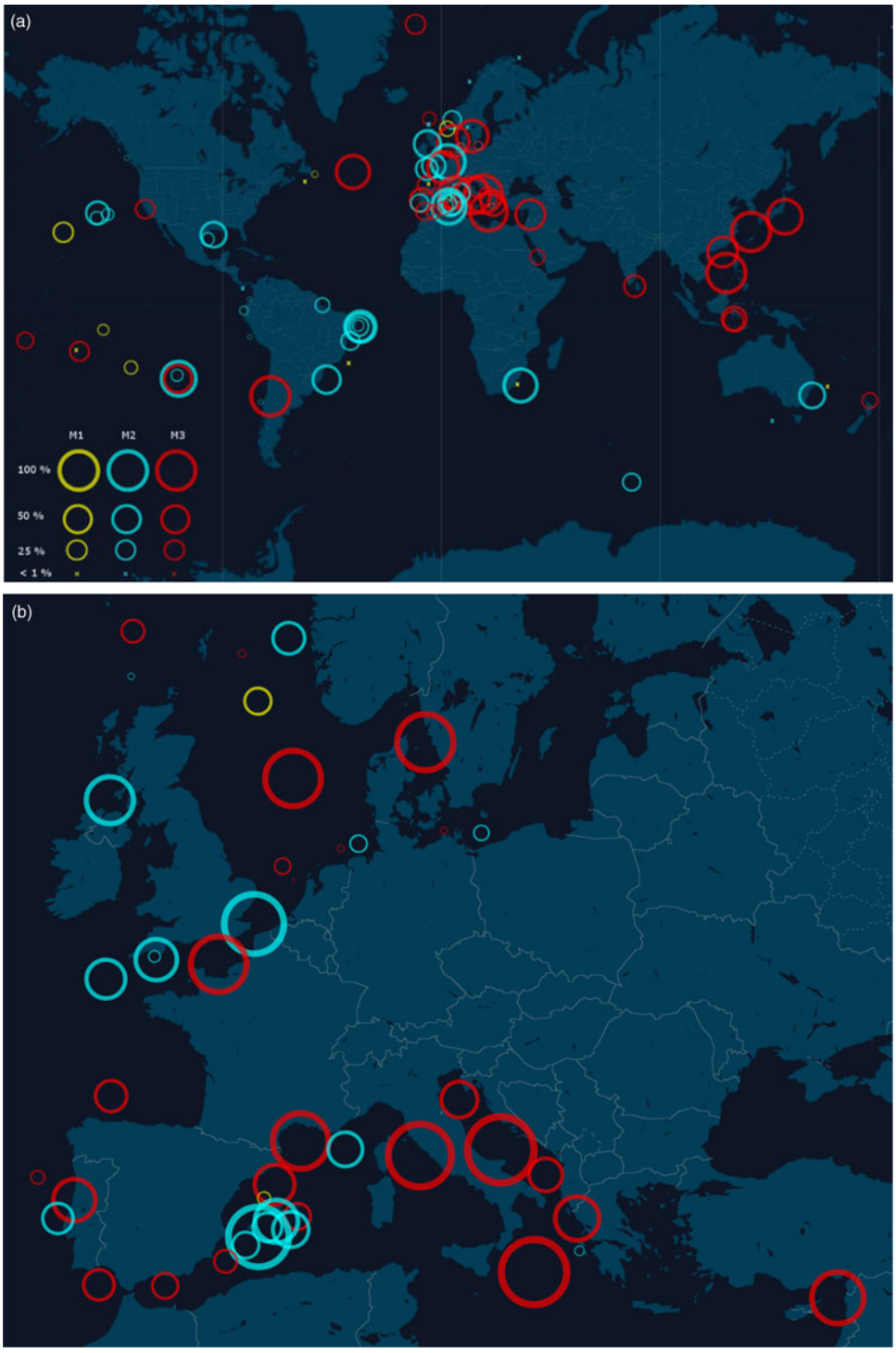

Figure 11. Bubble map of frequency of occurrence (FO) from plastic ingestion studies conducted with Method 1 (M1 – yellow), Method 2 (M2 – blue), and Method 3 (M3 – red) a) across the globe and b) in European waters (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis).

Table 4. Geographic distribution of research effort including only studies on plastic ingestion by fish.

Region	No. of studies	No. of examined species (#)	No. of examined specimens (#)
Atlantic North	28	161	125,515
Atlantic South	9	83	4794
Mediterranean	19	77	7033
Indian Ocean	7	56	16,072
Pacific North	10	71	2500
Pacific South	8	84	1937
Total	81	532	157,851

Note: The number of studies and species defers from the total number of studies (79) and species (475) examined, because some studies had sampling location in different regions, and some species were assessed in multiple studies and multiple regions. All three methods are included.

in surface water samples do not increase over time potentially due to 'loss' of plastic debris through fragmentation, sinking due to biofouling, shore deposition and ingestion by marine organisms. However, increasing trends of plastics concentrations in sediment core samples indicate that plastic pollution in fact is in rise (Matsuguma et al., 2017).

3.6. Fisheries interest and conservation status

Of 494 species examined in all 93 papers, 391 were of commercial importance. Of these 391 species, plastic ingestion was recorded in 262 species. Thus, 67% of examined commercially important species have been reported to have eaten plastics. Particularly high FO, excluding assessments with low sample sizes ($N < 10$), were reported in multiple species of commercial fish from coastal waters of China (all species 100%) (Jabeen et al., 2017), in gilthead seabream (*Sparus aurata*, Sparidae) (100%), golden gray mullet (*Chelon auratus*, Mugilidae) (95%) and common sole (*Solea solea*, Soleidae) (95%) from the Adriatic Sea (Anastasopoulou et al., 2018; Pellini et al., 2018) and in red mullet (*Mullus barbatus*, Mullidae) (92%) from Ionian Sea (Piccardo, Felline, & Terlizzi, 2018). In total, 49 species of commercial fish, examined with Method 3 and sample sizes over 10 ($N \geq 10$) had more than half of the individual fish (FO $\geq$ 50%) reported to contain plastic debris (Figure 12). From the conservation perspective, plastic ingestion occurred in 29 fish species with IUCN conservation status of increased vulnerability (threatened or near threatened) (Table 5).

3.7. Habitats and trophic levels

With respect to habitats, we found that most commonly examined species were collected from coastal benthic and oceanic pelagic habitats. Some habitat categories were under-represented, such as oceanic benthic and benthopelagic (Table 6), where the concentrations of microplastics are predicted to be the greatest (Kooi, van Nes, Scheffer, & Koelmans, 2018), most

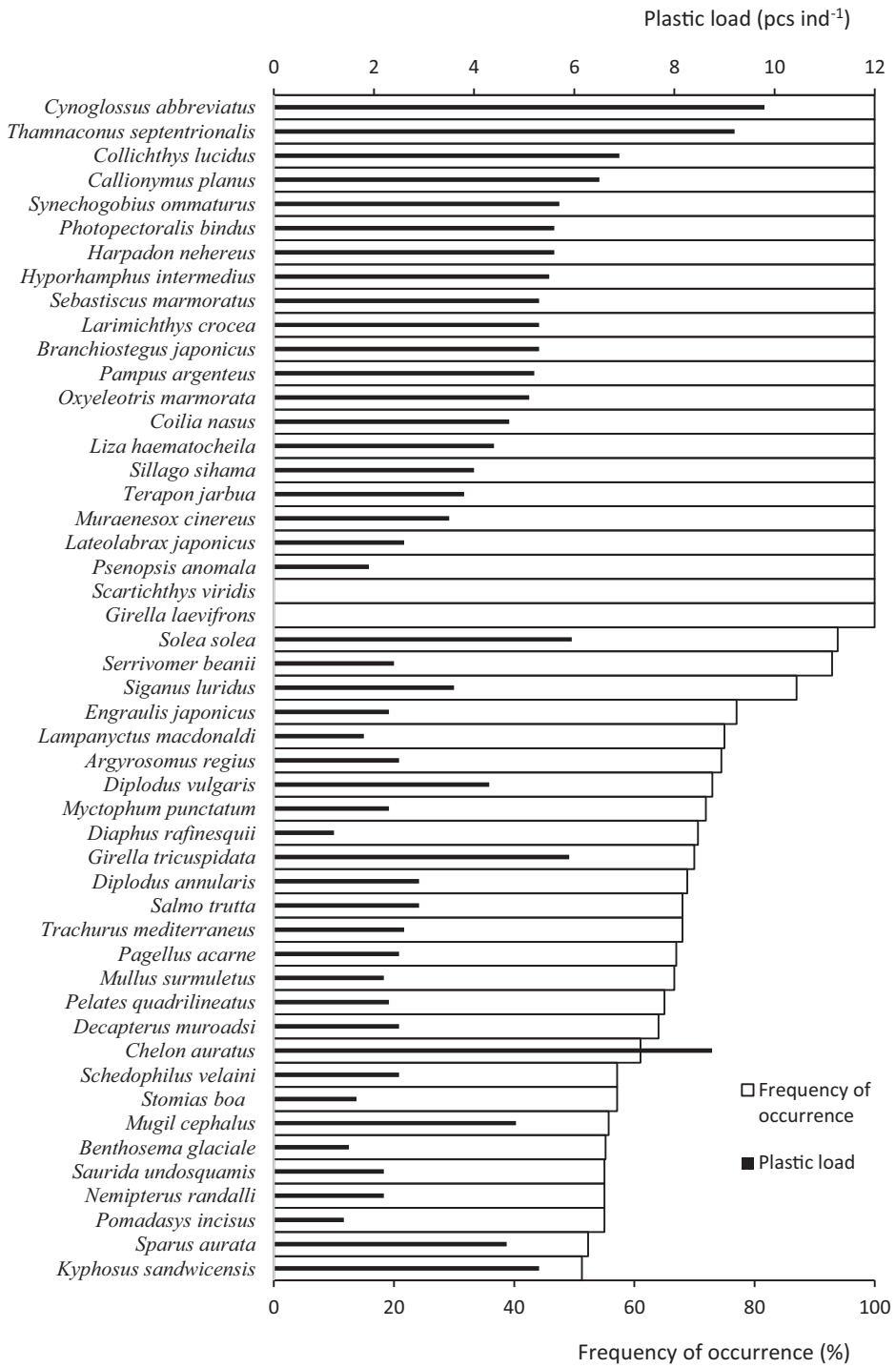

Figure 12. Frequency of occurrence (%) and plastic load (pc ind^{-1}) in 49 commercial fish species. Only assessments done with Method 3, $N \geq 10$ and FO $\geq$ 50% are displayed. The FO and PL for species assessed in multiple independent studies was calculated as the mean, weighted and arithmetic, respectively, of all the assessments of one species done with Method 3 (gut content digestion analysis).

Table 5. Fish species with a record of plastic ingestion listed on IUCN Red List based on the global assessment.

IUCN status	Species	Common name	Reference
CR	*Thunnus maccoyii*	Southern bluefin tuna	Young et al., 1997
	Anguilla anguilla	European eel	Steer et al., 2017
EN	*Sphyrna lewini*	Scalloped hammerhead	Cliff et al., 2002
	Thunnus thynnus	Atlantic bluefin tuna	Romeo et al., 2015
VU	*Isurus oxyrinchus*	Shortfin mako shark	Cliff et al., 2002
	Carcharodon carcharias	Great White shark	Cliff et al., 2002
	Sphyrna zygaena	Smooth hammerhead	Cliff et al., 2002
	Carcharhinus obscurus	Dusky shark	Cliff et al., 2002
	Carcharias taurus	Sand tiger shark	Cliff et al., 2002
	Lamna nasus	Porbeagle	Joyce et al., 2002
	Rhincodon typus	Whaleshark	Haetrakul et al., 2007
	Gadus morhua	Atlantic cod	Foekema et al., 2013
	Thunnus obesus	Bigeye tuna	Choy and Drazen 2013
	Melanogrammus aeglefinus	Haddock	Foekema et al., 2013
	Alopias superciliosus	Bigeye thresher	Benjamin et al., 2014
	Lutjanus campechanus	Northern red snapper	Phillips and Bonner 2015
	Squalus acanthias	Picked dogfish	Avio et al., 2015
NT	*Galeocerdo cuvier*	Tiger shark	Cliff et al., 2002
	Carcharhinus leucas	Bull shark	Cliff et al., 2002
	Carcharhinus limbatus	Blacktip shark	Cliff et al., 2002
	Carcharhinus brachyurus	Bronze whaler	Cliff et al., 2002
	Thunnus alalunga	Albacore	Romeo et al., 2015
	Paralichthys lethostigma	Southern flounder	Phillips and Bonner 2015
	Centroscymnus coelolepis	Portuguese dogfish	Cartes et al., 2016
	Lutjanus synagris	Lane snapper	Vendel et al., 2017
	Sciaena umbra	Brown meager	Güven et al., 2017
	Thunnus albacares	Yellowfin tuna	Markic et al., 2018
	Prionace glauca	Blue shark	Bernardini et al., 2018
	Lutjanus analis	Mutton snapper	Pegado et al., 2018

Note: CR, critically endangered; EN, endangered; VU, vulnerable; NT, near threatened.

Table 6. Research effort across different habitats, based on plastic ingestion studies and including all three methods.

	Neritic	Neritic-oceanic	Oceanic	Total
Pelagic	69	32	89	190
Bentho-pelagic	77	24	6	107
Benthic	270	64	14	348
Total	416	120	109	645

Note: The values show the number of assessments per vertical and horizontal component of the habitat.

likely because these habitats are less accessible than others. We were not able to compare the FO across different habitats due to insufficient data obtained by Method 3 for all habitat categories.

The distribution of the trophic levels of examined fish varies across the three methods (Figure 13a). Method 1 was mainly used for fish with trophic levels over 3, while Method 3 encompassed a wider range of trophic levels, from 2 to 4.5. Fish body size is inversely related to trophic levels (Pauly, Christensen, Dalsgaard, Froese, & Torres, 1998), meaning that the fish from lower trophic levels are generally smaller than the fish from higher trophic levels. This would agree with Method 1 being mainly used to examine larger fish, while Method 2 and 3 being applied on

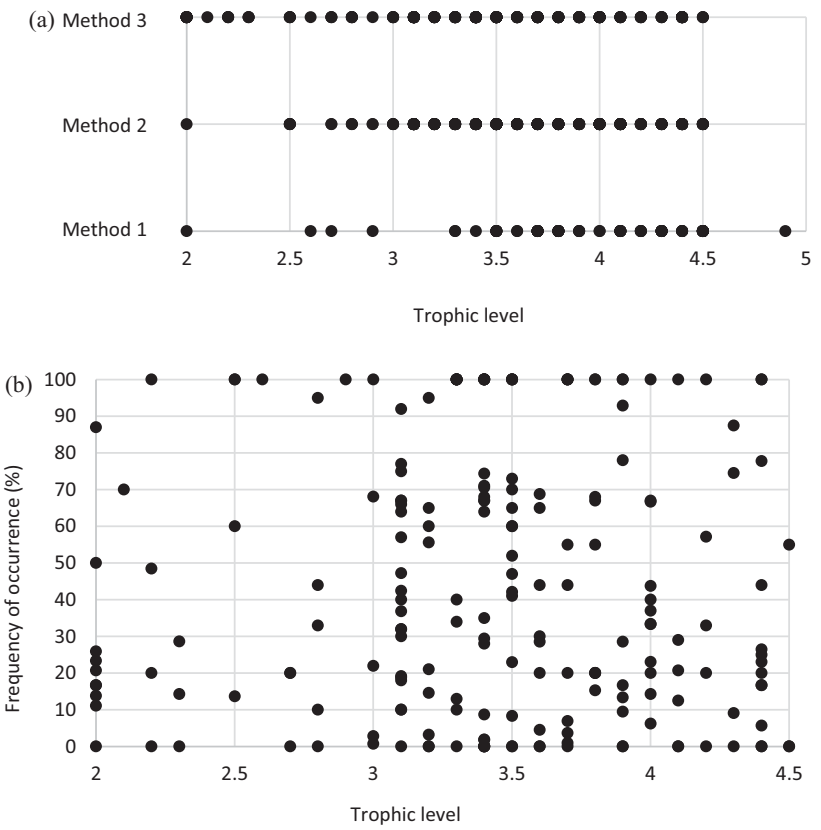

Figure 13. a) Representation of trophic levels of fish species examined with three different analytical methods (Method 1 – naked-eye examination, Method 2 – microscopic analysis, Method 3 – gut content digestion analysis), and b) scatter plot of frequency of occurrence with respect to trophic levels of examined species analyzed with Method 3.

generally smaller fish. Furthermore, no relationship was found between FO and trophic levels ($p = 0.671$) (Figure 13b). Spearman's rank correlation coefficient on assessments analyzed using Method 3 alone was $R = -0.030$. Due to insufficient data for some trophic guilds, we did not compare the FO among the guilds.

Reviewed plastic ingestion studies which investigated the differences in FO among habitats used variable categorization and reported inconsistent results, which makes it difficult to bring unanimous conclusion on the overall patterns. However, it seems that pelagic species were reported slightly more often to exhibit higher FO than species from other habitats. Güven et al. (2017) found significantly more plastic ingested in the neritic-pelagic zone than others. Pelagic species examined by Rummel et al. (2016) and Digka, Tsangaris, Torre, Anastasopoulou, and Zeri (2018) also contained significantly more plastic debris than demersal species. Anastasopoulou et al. (2018) found more macroplastics in pelagic fish than in mesopelagic and demersal, but with respect to microplastics there was no significant difference among habitats. Markic et al.

(2018) found benthopelagic fish to ingest plastic significantly more often than pelagic and benthic and demersal, while no difference was found with respect to the horizontal distribution of the species. Lusher et al. (2013) and Neves et al. (2015) did not find significant difference.

Fewer studies examined the occurrence of plastic ingestion with respect to trophic levels and feeding strategies. In accordance with our findings, none of the studies found significant relationship between the trophic levels and plastic ingestion (Güven et al., 2017; Forrest and Hindell 2018; Markic et al., 2018; Pegado et al., 2018). In contrast, with respect to trophic guilds, categorization and findings were more inconsistent. Mizraji et al. (2017) and Markic et al. (2018) found the greatest plastic ingestion in omnivorous species, while conversely, Jabeen et al. (2017) reported the least plastic debris in omnivores, as opposed to carnivores and planktivores. Miranda & de Carvalho-Souza (2016) found plastic debris only in two carnivorous species out of 11 species of various trophic guilds. Phillips and Bonner (2015) recorded the greatest ingestion in pelagic carnivores, but they did not examine omnivorous fish or herbivorous fish. Due to such variability little can be deduced from the results on the patterns of plastic ingestion across trophic guilds, However, it is safe to say that there is no apparent relationship between trophic level and occurrence of plastic ingestion in marine fish.

3.8. Concerns related to plastic ingestion

There are 6,563 commercial marine and diadromous fish species (FAO, 2016) and only 7.5% have been examined for plastic ingestion and reported to date. However, exposure of fish to plastic pollution through ingestion is evident. Of the 391 commercial species examined in the reviewed studies, 67% were reported to contain plastic. From anthropocentric point of view, this brings seafood safety into question (Rochman et al., 2015). The results of our study urge the need for more research to fully understand the complex chemical and biochemical interactions between marine organisms, organic pollutants and plastic debris and their potential impacts on human health (Van Cauwenberghe & Janssen, 2014; Koelmans, 2015; Ziccardi, et al., 2016; Barboza, Vethaak, Lavorante, Lundebye, & Guilhermino, 2018).

3.9. Recommendations

Based on the findings of our review, we compiled the following recommendation list:

1. Sample size: Due to the evident sample size bias and underestimation of plastic ingestion with low sample sizes, for more robust and statistically

Table 7. Recommended sample sizes for future studies on plastic ingestion by fish based on FO values obtained in this review (FO = 38%, $p = 0.38$), with confidence level of 95% and margin of error 10% and 20%.

	Margin of error 10%	Margin of error 20%
Confidence level 95%	$N \geq 91$	$N \geq 23$

sound surveys, we recommend a sample size of a bare minimum of 10 specimens per assessment. Furthermore, based on the global FO obtained with Method 3 (i.e. FO = 38% equals to the estimated population mean $P = 0.38$), which is characterized with a low standard error (0.5%), we provided minimum sample sizes for future studies for confidence level of 95% and margin of error of 10% and 20% (Table 7), as the first estimate for a sample size. Thus, depending on the research question, satisfying confidence level and acceptable margin of error, required sample sizes should be calculated prior to sample collection.

2. Analytical method: It is understandable that the choice of analytical methods will depend on resource and time constraints. However, we do suggest choosing a more in-depth analysis capable of detecting microplastics over naked-eye examination, even with copious amounts of gut content. Results of Method 1 showed that even very large sample sizes ($N > 1000$) yielded very low FO, thus lowering the sample size and applying Method 3 would be more efficient. For isolation of plastics, we suggest chemical or enzymatic digestion of the gut content and subsequent filtration with a fine mesh. From our own experience, if the gut content is well digested, filtration with a 50 µm mesh should not pose a problem. Karami, Golieskardi, Choo, et al. (2017) proposed the use of 10% KOH at 40 °C for chemical digestion, which in their method testing proved to be the most efficient for digestion of the gut content and the least destructive for plastics. Before the start of the laboratory analysis, the chosen method should be tested on a test sample spiked with test microplastics, which, if the method is valid, should all be retrieved.
3. Polymer identification: If identification of retrieved microplastics is uncertain, the suspicious particles should be subjected to a chemical analysis by FTIR or Raman spectroscopy (Hermsen et al., 2018). Additionally, the person examining the sample should be trained in detecting plastics under a microscope to minimize misidentification.
4. Digestive system: The entire gastro-intestinal tract should be examined, not just the stomach (Jabeen et al., 2017; Hermsen et al., 2018). Additionally, diet analysis should also be performed to determine feeding ecology of examined species.
5. Secondary ingestion: If any undigested prey is found in the gut content, they should be examined for plastic ingestion as well.

6. Exceptional care should be taken regarding contamination, particularly airborne contamination by fibers, or loss of microplastics. However, fibers should not automatically be excluded from analysis, as they present an important component of marine plastics whose ingestion by marine fish should be documented (Hermsen et al., 2018). Additionally, laboratory blanks should be used as a control method for airborne contamination.

4. Conclusion

Plastic ingestion has been confirmed in 323 marine fish species from around the globe, of which 262 (81%) are of commercial importance. The detection of plastic largely depends on the sampling effort and analytical methods, so these numbers would most likely be greater if more suitable methodology was applied. We found that the detection of plastic ingestion was positively correlated with increased sample sizes (up to $N = 10$). We also found that the analytical methods in which the chemical digestion of the gut content was used detected plastic ingestion significantly more often than the visual methods (naked-eye and microscopy). Furthermore, due to multiple methodological bias, it would not be sound to draw firm conclusions regarding the patterns in the occurrence of plastic ingestion in various geographic regions, and across a range of habitats, trophic levels and groups. However, we do know plastic ingestion is common in fish and that it can potentially cause adverse physiological changes in fish. As global fisheries depend on healthy fish and oceans, plastic pollution is certainly becoming a major challenge for this industry. Plastic ingestion by fish is also raising great concerns due to potential risks to human health, yet there is still very little information on plastic ingestion in commercial fish species, as only 7.5% have been examined. Many efforts have been put into mitigating plastic pollution, including awareness raising and beach clean-ups, research, monitoring and improving waste management. Several experts even suggested that plastic waste should be classified as hazardous (Rochman, Browne, et al., 2013) and treated as persistent organic pollutants (Worm et al., 2017). To ensure we are tackling the issue from all corners, more work is needed on market-based instruments and policies, regulations and legislation, as well as measuring their positive impacts and further increasing awareness (Rochman, Cook, & Koelmans, 2016; Xanthos & Walker, 2017), with a special empasis on prevention of pollution, since microplastics cannot be remediated from the marine environment.

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